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CHARACTERISTICS AND REGULATION OF FISSION ACTIVITY IN CLONAL CULTURES OF THE COSMOPOLITAN SEA ANEMONE, *HALIPLANELLA LUCIAE* (VERRILL) [1]

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The diversity of reproductive modes within the Cnidaria is greater than that of most other metazoan groups. This is particularly evident among the sea anemones, for which Chia (1976) has compiled a list of reproductive modes, and has speculated on their evolution and adaptive significance. Reproduction in *Haliplanella* (= *Diadumene*) *luciae* Verrill is of special interest because *H. luciae* has greatly expanded its geographical range since the turn of the century (Uchida, 1932; Stephenson, 1935; Hand, 1955; Shick and Lamb, 1977). This species now occurs intertidally on boreal (Uchida, 1936; Williams, 1973), temperate (Stephenson, 1935; Hand, 1955) and tropical (Dunn, personal communication; Belem and Monteiro, 1977) coasts.

The ability of *H. luciae* to establish new populations is extraordinary since, unlike other intertidal invertebrates, *H. luciae* has never been observed to produce larvae as agents of dispersal (Davis, 1919; Shick, 1976). All observed reproduction has been asexual, through longitudinal fission (Hargitt, 1912; Davis, 1919), and less commonly by pedal laceration (Atoda, 1954; Johnson and Shick, 1977). Although a single fission event infrequently produces multiple individuals, most fission events are binary, and analogous to cytokinesis (Atoda, 1976; Minasian, 1976).

Longitudinal fission permits the rapid establishment of intertidal clones (Chia, 1976; Francis, 1976). The strategic advantages of fission have been recently discussed by Francis (1976) and Shick and Lamb (1977). Hoffmann (1976) and Shick and Lamb (1977) described the genetic composition of different conspecific clones of anemones, and have made valuable inferences concerning advantages of asexual reproduction in contrast to sexual reproduction.

Understanding the contribution of asexual reproduction to the success of *H. luciae* makes necessary the analysis of asexual reproductive rates and their regulation. However, few studies have evaluated asexual reproductive rates of sea anemones, partly due to a lack of standardized methods.

Researchers of hydroid development have calculated exponential rates of increase (symbolized "k") in numbers of polyps in laboratory cultures (Loomis, 1954), and have related these rates to environmental variables (Fulton, 1962; Davis, 1971). Minasian (1976) determined k (fission rate) in laboratory populations of *H. luciae*, and quantified the effect of feeding frequency on k. The occurrence of fission in *H. luciae* is affected by temperature (Miyawaki, 1952) and fluctuating

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temperature-emersion treatments (Johnson and Shick, 1977); but the effect of temperature on k was not quantified in those studies.

The present study quantifies the combined effects of temperature and feeding frequency on k in *H. luciae*, and provides a comprehensive analysis of longitudinal-fission activity in a sea anemone. This includes previously undescribed characteristics of fission activity, essential to the understanding of the regulation of longitudinal fission. In addition, we describe a method for establishing and maintaining permanent *in vitro* clonal cultures of *H. luciae*. This has enabled us to obtain a range of possible values of k in a clone of *H. luciae* from northwestern Florida.

Materials and Methods

Establishment of clonal cultures

Specimens of *H. luciae* were collected on 2 October from a sand-flat intertidal region adjacent to the Florida State University Marine Lab near Turkey Pt., on the N. W. Florida Gulf coast. These anemones occur on the undersides of sedimentary stones, accompanied by barnacles (*Chthamalus fragilis*) in the upper littoral region, and oysters (*Crassostrea virginica*) in the mid-littoral region. *H. luciae* cloned from this location (29° 54.8′ N, 84° 30′ W) have a brownish-green column with 12 to 24 yellowish-orange longitudinal stripes in large individuals. Shick and Lamb (1977) found a sample of striped *H. luciae* from this population to consist of two different genotypes (*i.e.* clones). The specimens of *H. luciae* cloned in this study are presumed to belong to the same genotype, since they are phenotypically (sex and color) identical and phenotypically distinguishable from the second striped clone present at the collection site.

To establish clonal cultures, three individual anemones of average diameter (about 5 mm) were isolated in three covered glass storage dishes (Corning #3250), each containing approximately 200 ml natural sea water (28 to 30‰). These cultures were maintained at room temperature (22 to 25° C) under a 14/10 hr light-dark cycle provided by a fluorescent (cool-white) light source, and fed to repletion (30 to 60 min) with newly hatched *Artemia* nauplii every second day. All culture dishes were rinsed briefly with distilled water and refilled with fresh sea water daily. The number of anemones in each culture increased rapidly through longitudinal fission.

After 3 months each clonal culture was divided into two cultures, producing a total of six clonal cultures. These served as stock cultures for the experiments, and were kept under the same photoperiod at a temperature of 16 to 18° C; feeding frequency was reduced to twice per week. Every 4 to 6 weeks each clonal culture was transferred to clean glassware. Clonal cultures were maintained under this standardized regimen for at least 4 months before being used in experiments. Anemones from stock cultures were less than 7 mm in diameter; histological examination showed that none of these anemones bore gonads.

Culture experiments

For each experiment, six duplicate cultures were set up by placing 10 anemones from each stock culture in glass bowls (glass stacking dish, Wheaton #350134)

which were 11.5 cm in diameter, and contained approximately 150 ml of sea water. Sets of six cultures were then placed under one of nine different experimental regimens. These regimens, evaluating combined temperature and feeding effects upon fission activity, were as follows: fed once every 2 days at 26, 21 or 16° C; fed once every 4 days at 26, 21 or 16° C; starved at 26, 21 or 16° C. Thus the entire experiment involved a total of 54 clonal cultures, each initially containing 10 anemones. In addition, fission activity in stock cultures was evaluated by setting up six experimental cultures as above, and observing fission activity for 3 months under maintenance conditions (fed twice per week at 16 to 18° C). Experimental cultures were exposed to the new temperature for 2 days before beginning the experiment, providing time for attachment of anemones to culture bowls. The second day after transfer to culture bowls (day 0) marked the start of the experiment; for experimental groups receiving food, day 0 was the day of the first feeding. These cultures were also under a 14/10 hr light-dark cycle. Anemones were counted daily, at which time they were fed, if necessary, and the sea water changed.

Statistical analysis

Fission rates were calculated from the least-squares regression equation of the natural log number of anemones as a function of time in culture: $\ln N_i = kt_i + \ln N_o$. Here the rate of increase in log number of anemones ($\ln N_i$) on a given day (t_i) is defined by the fisson rate (k), which is the slope of the regression line. The number of polyps present at the start (day 0) of the culture interval, and Y-intercept of the regression line, is $\ln N_o$. This equation was employed by Loomis (1954) and Fulton (1962) to calculate the exponential rate of increase (k) for asexual production of hydroid polyps, in which $N_i = N_o e^{kt_i}$.

Since exogenous factors can impose a variable delay period prior to the initiation of fission, it was necessary to recognize two distinct parameters for fission rate, defined as follows. The overall fission rate (k) is the rate of increase during the entire culture interval, including the initial delay before the onset of fission. The second parameter, k_{adj}, gives the rate of increase subsequent to the initial delay period. Adjusted fission rate (k_{adj}) is calculated by the same method as k, except that the first data entry, day 0, is the day prior to the first occurrence of fission. Hence, k_{adj} designates the rate of increase only after the initiation of fission, rather than during the entire culture period, and better estimates the rate of active, sustained fission. Where the delay before onset of fission is absent or very short, $k = k_{adj}$. Both k and k_{adj} were calculated for each culture, except where fission activity was not sustained and considered to be nonexponential. The delay before the onset of fission also was calculated for each culture.

The effects of temperature and feeding on k and k_{adj} were evaluated by means of a two-way analysis of variance (ANOVA). Paired comparisons between statistical means were performed using t-tests.

The percentage of anemones undergoing fission per day was calculated as follows $(N_i - N_{i-1}) \cdot 100/N_i$, where N_i is the number of anemones on a given day, and N_{i-1} is the number present on the previous day. An angular transformation was performed on these percentage data, and means and standard deviations

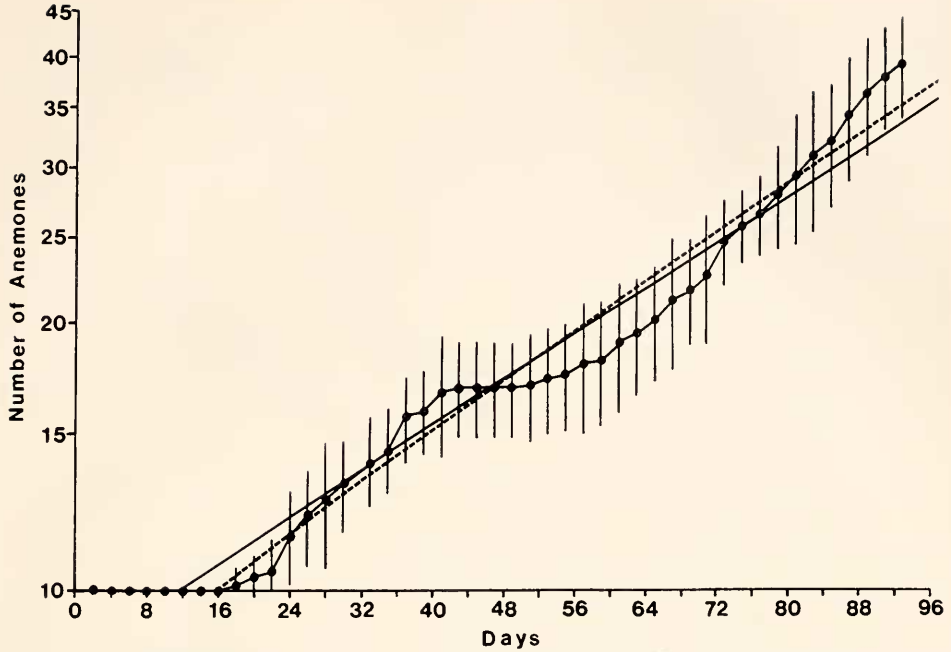

FIGURE 1. Semilogarithmic plot of numerical increase through longitudinal fission in cultures of *H. luciae*, reared under conditions of routine maintenance ($17 \pm 1°$ C, fed twice per week). Day 0 is 2 days after transfer to culture bowls from stock cultures. Each point is a mean of six cultures, each intially containing 10 anemones; error bars are standard deviations. The slope of the regression equation, $\ln N_t = kt_1 + \ln N_o$, is the fission rate, k (see Materials and Methods for further explanation). The slope of the solid line (k) is 0.0149 ± 0.0017 (mean $\pm$ s.d.); the slope of the broken line, k_{adj} (0.0162 ± 0.0010), is corrected for the initial delay period prior to the start of fission activity.

calculated for each regimen. On plots of the temporal pattern of fission pulses, the scale of the ordinate conforms to the transformation, whereas the units on the ordinate are actual, untransformed percentages. This transformation is necessary to assume normality (Sokal and Rohlf, 1969). Means and standard deviations were calculated for relative maxima of all fission pulses (pulse maxima), and for relative minimal values between fission pulses (pulse minima); these were evaluated using a multiple-range (Student-Neuman-Keuls) test. Lengths of intervals between fission-pulse maxima were also determined. A G-test for independence (Sokal and Rohlf, 1969) was performed on frequency data for pulse intervals. Test statistics corresponding to probabilities of less than 0.05 were regarded as significant.

RESULTS

Characteristics and exogenous regulation of fission activity

Figure 1 illustrates prominent characteristics of fission activity in clonal cultures reared under the standard maintenance regimen. These characteristics

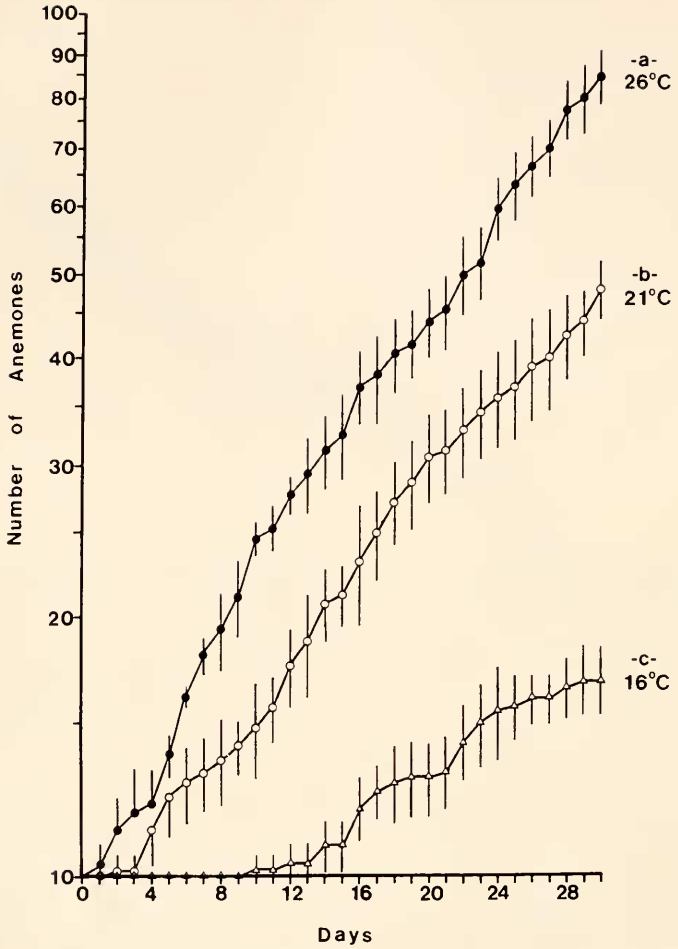

FIGURE 2. Semilogarithmic plot of numerical increase through longitudinal fission in cultures of *H. luciae* fed once every 2 days. Each point represents a mean of six cultures, each initially containing 10 anemones; error bars are standard deviations. Three experimental culture temperatures were used: (a), 26° C; (b), 21° C; (c), 16° C.

included: first, an initial delay period before the onset of fission; second, an exponential fission rate followed by a temporary cessation of fission; and third, resumption of the exponential fission rate. The variable delay lasted for 21.33 ± 4.46 (mean $\pm$ s.d.) days, although one culture initiated fission as early as day 18 (Fig. 1). This delay was initiated by the mechanical disturbance imparted in transferring anemones to new culture bowls. For the entire culture interval of 92 days, $k = 0.0149 \pm 0.0017$ (mean $\pm$ s.d.); with the delay period omitted from calculation, $k_{adj} = 0.0162 \pm 0.0010$. The calculation for k_{adj} usually increased the estimate of fission rate in addition to shifting the regression line to the right.

Figures 2 and 3 demonstrate the numerical increase in cultures of *H. luciae*

which were fed *Artemia*. At 26° C little or no delay occurred prior to the onset of fission; hence k and k_{adj} were essentially identical. Only when anemones were fed once every two days at 16° C was k_{adj} significantly larger than k (t-test, $P < 0.05$).

Values of k_{adj} in experimental cultures ranged from 0.0278 (doubling time = 24.9 days) at 16° C to 0.0728 (doubling time = 9.5 days) at 26° C, in cultures fed every second day. Analyses of temperature and feeding effects showed both factors to significantly affect k and k_{adj}, based upon comparisons between 21° and 26° C regimens (Table I). Temperature coefficients (Q_{10}) for k_{adj} over the 21 to 26° C temperature range were 1.6042 and 1.6759 for feeding frequencies of 2 and 4 days, respectively (Table II). Thus, the difference in feeding frequency did not influence the effect of temperature upon k_{adj} in this range. Since the Q_{10} for the 16 to 21° C range was much larger (Table II), the greatest effect of temperature on k_{adj} occurred below 21° C.

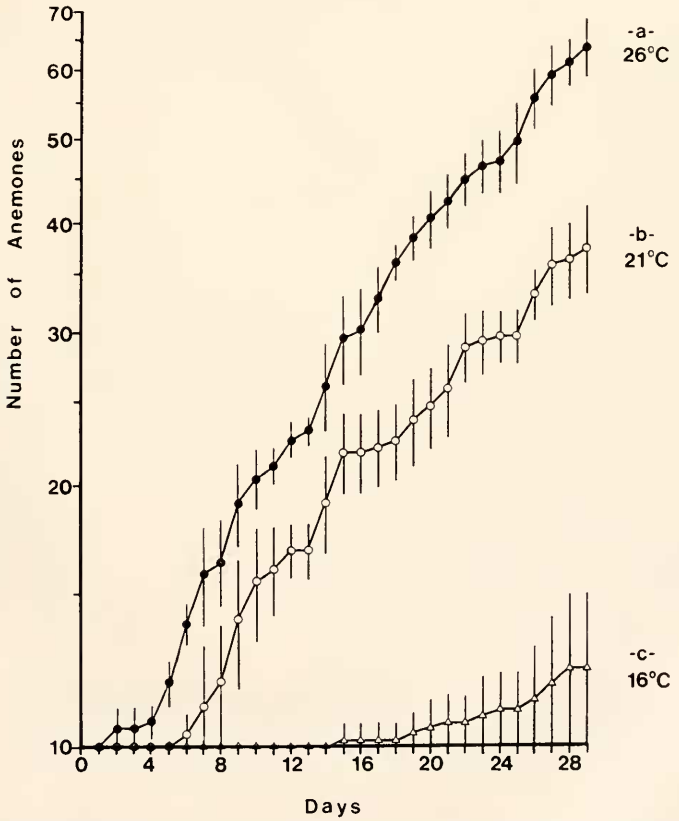

FIGURE 3. Semilogarithmic plot of numerical increase through longitudinal fission in cultures of *H. luciae* fed once every 4 days. Each point represents a mean of six cultures, each initially containing 10 anemones; error bars are standard deviations. Three experimental culture temperatures were used: a), 26° C; (b) 21° C; (c), 16° C.

TABLE I

Means ± standard deviations for values of k *and* k_{adj} *for* H. luciae *reared at different temperatures and feeding frequencies. Each experimental mean consisted of six bowls, each initially containing 10 anemones. The resulting two-way ANOVA was performed on four means (21° vs. 26° C, fed every second or fourth day). The ANOVA results were the same for both* k *and* k_{adj}*, and indicate if an effect upon* k *was statistically significant (+) or not significant (−) at the 5% level; n.c. = fission activity not sufficient to calculate* k.

Feeding frequency	Temperature		
	16 ± 1° C	21 ± 1° C	26 ± 1° C
	Values of k		
2 days	0.0207 ± 0.0036	0.0569 ± 0.0040	0.0728 ± 0.0035
4 days	n.c.	0.0518 ± 0.0031	0.0695 ± 0.0032
	Values of k_{adj}		
2 days	0.0278 ± 0.0047	0.0574 ± 0.0035	0.0727 ± 0.0036
4 days	n.c.	0.0533 ± 0.0045	0.0690 ± 0.0025

ANOVA: Effects for 21° vs. 26° C

Temperature	Feeding	Interaction
(+)	(+)	(−)

Both temperature and feeding significantly affect length of the delay period prior to the initiation of fission (Table III). The duration of delay periods varied from 1 day or less at 26° C, to over 21 days at 16° C. Table III shows that decreasing the feeding frequency from 2 to 4 days further lengthened this delay. Temperature and feeding frequency acted synergistically upon the pre-fission delay, as indicated by the significant ANOVA interaction term (Table III). For example, at 26° C halving the feeding frequency (from every second to every fourth day) increased the mean delay period by 1.3 days; at 16° C, halving the feeding frequency increased the delay by over 10 days (Table III).

Fission activity in starved cultures at 26° C and 21° C was limited to two major pulses of fission activity (Fig. 4); starved anemones at 16° C did not

TABLE II

Temperature coefficients (Q_{10}) *for fission rate* (k_{adj}) *in* H. luciae *at two different feeding frequencies; n.c. = insufficient data for calculation.*

Feeding frequency	Temperature Range		
	16°–21° C	21°–26° C	16°–26° C
2 days	4.3567	1.6042	2.6436
4 days	n.c.	1.6759	n.c.

TABLE III

Means ± standard deviations for delay to onset of fission (days) in H. luciae *at different temperatures and feeding frequencies. Each experimental mean consisted of six bowls, each initially containing 10 individual anemones. The two-way ANOVA result indicates if an effect on fission delay is statistically significant (+) or not significant (−) at the 5% level.*

Feeding frequency	Temperature		
	16 ± 1° C	21 ± 1° C	26 ± 1° C
2 days	11.6667 ± 2.3381	1.8333 ± 0.9832	0.0000 ± 1.0954
4 days	>21.0	5.5000 ± 1.3784	1.3333 ± 1.5055

ANOVA: Effects for 21° vs. 26° C

Temperature	Feeding	Interaction
(+)	(+)	(+)

undergo fission. Nonetheless, these cultures remained healthy throughout the experiment.

Temporal pattern of fission activity

After long delay periods a major pulse of synchronous fission occurred, followed by a temporary cessation of fission (Figs. 1, 2c). The resumption of fission after this initial pulse was either less synchronous and without additional major plateaus (Fig. 1), or continued to show additional, brief cessations of fission (Fig. 2c).

H. luciae exhibited distinct pulses of increased fission activity, with relative maxima (peaks) and minima (troughs), shown in Figures 5 and 6. These occurred

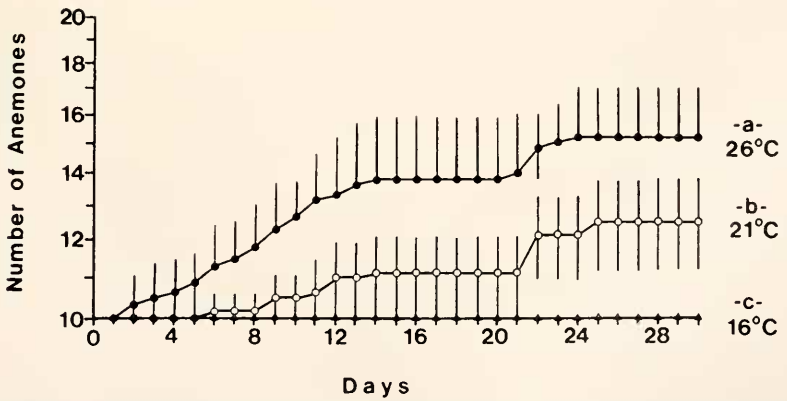

FIGURE 4. Semilogarithmic plot of numerical increase through longitudinal fission in starved cultures of *H. luciae*. Each point represents a mean of six cultures, each initially containing 10 anemones; error bars are standard deviations. Three experimental culture temperatures were used: (a), 26° C; (b), 21° C; (c), 16° C.

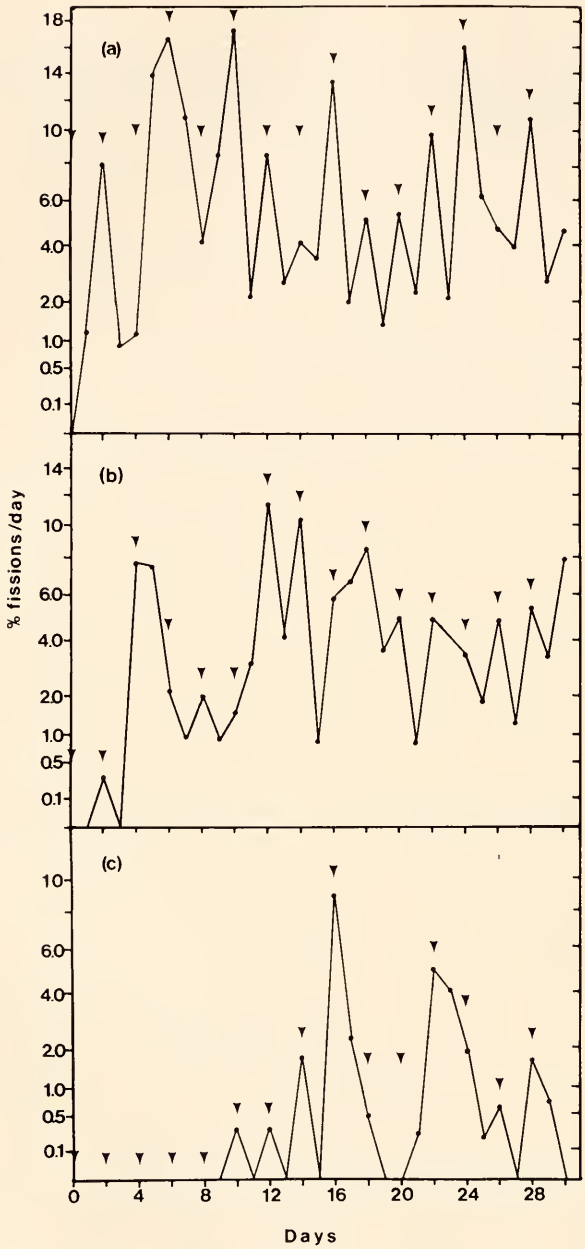

FIGURE 5. Percentages of *H. luciae* undergoing fission per culture per day in cultures fed once every 2 days. Each percentage is a mean of six cultures; arrowheads indicate feeding days. Data was subjected to angular transformation prior to statistical computations; plotted means are transformed data. The ordinate indicates actual, untransformed percentages. Three culture temperatures are represented: (a) 26° C; (b) 21° C; (c) 16° C.

TABLE IV

Different lengths of time periods between peak values of fission activity (pulse maxima), observed in H. luciae *culture populations. Cultures initially consisted of 10 anemones each and were reared under different temperature and feeding regimens for 1 month. Six clonal cultures were reared under each different culture regimen. The percentage of fissions occurring each day were then averaged, and the peaks of fission activity determined. Occurrence of the various lengths of pulse intervals depend upon culture conditions (G-test for independence, $P < 0.05$).*

Culture regimen	Length of period between pulse maxima (days)			
	2	3	4	>4
26° C, fed every 2 days	7	0	3	0
26° C, fed every 4 days	0	5	1	1
21° C, fed every 2 days	5	0	4	0
21° C, fed every 4 days	1	3	2	0
16° C, fed every 2 days	4	0	1	1

in cycles, with intervals between pulse maxima usually lasting for 2 to 4 days. These pulses of fission had a phasic dependence upon the feeding regimen. In cultures which were fed every second day, pulse maxima occurred only on feeding days, although not on all feeding days (Fig. 5). Therefore, periods between pulse maxima were multiples of 2 days, in cultures fed every second day. At 26° and 21° C most pulse maxima had a 2-day periodicity, with fewer pulse maxima being 4 days apart (Fig. 5a, b). In the 16° C cultures, a 6-day period between pulse maxima occurred at one point, although 2-day periods were still most frequent (Fig. 5c).

In cultures fed at 4-day intervals pulse maxima often occurred on days other than feeding days, and were usually 3 or 4 days apart (Fig. 6). Thus, the periodicity of pulse maxima was longer than that in cultures receiving food at

TABLE V

Means and standard deviations for fission-pulse maxima and minima observed in cultures of H. luciae *reared under different culture regimens. Each determination was taken from angular-transformed, averaged data from six clonal cultures. Means are for pulse maxima or minima occurring over a one-month period. Sample sizes are in parentheses. Asterisks denote means which are significantly different from all other means in the same column (Student-Neuman-Keuls test, $P < 0.05$).*

Culture regimen	Pulse maximum†	Pulse minimum†
26° C, fed every 2 days $k_{adj} = 0.0727$	18.359 ± 4.601 (11)	8.823 ± 1.893 (11)*
26° C, fed every 4 days $k_{adj} = 0.0690$	16.967 ± 5.039 (8)	6.486 ± 3.790 (8)
21° C, fed every 2 days $k_{adj} = 0.0574$	13.393 ± 5.014 (10)	6.828 ± 3.467 (10)
21° C, fed every 4 days $k_{adj} = 0.0533$	15.602 ± 7.650 (8)	3.658 ± 3.226 (8)*
16° C, fed every 2 days $k_{adj} = 0.0278$	7.920 ± 5.390 (7)*	0.417 ± 1.021 (6)*

† angular transformation (arcsin-$\sqrt{\%}$ fissions/day) is necessary to assume normality.

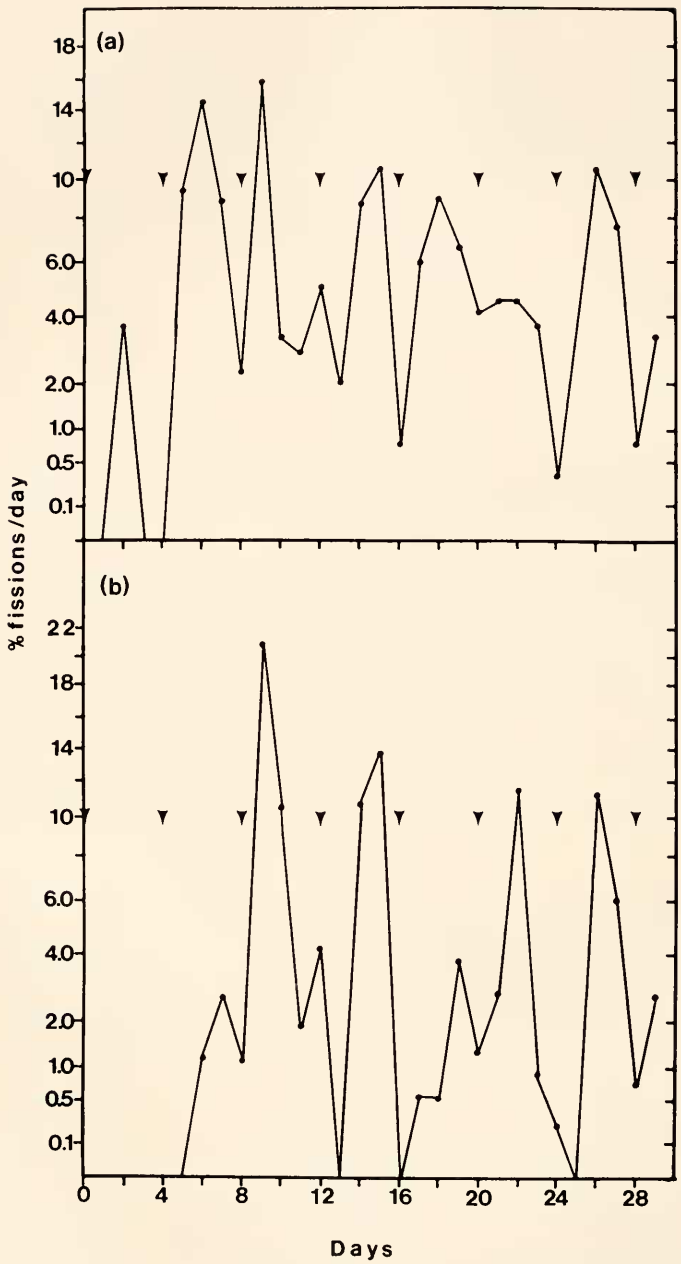

FIGURE 6. Percentages of *H. luciae* undergoing fission per culture per day in cultures fed once every 4 days. Each percentage is a mean of six cultures, each containing 10 or more anemones; arrowheads indicate feeding days. Data was subjected to angular transformation prior to statistical computations; plotted means are transformed data. The ordinate indicates actual, untransformed percentages. Two culture temperatures are represented: (a) 26° C; (b) 21° C.

2-day intervals. Only one period of 2 days was observed at the 4-day feeding frequency (Fig. 6b). Table IV summarizes the occurrence of pulse periodicity, which is dependent upon culture conditions (G-test, $P < 0.05$).

Pulse maxima often attained 10 to 20% of the culture population undergoing fission per day (untransformed percentages are given on the ordinate in Figs. 5 and 6). Pulse minima usually dropped below 4% fissions per day in cultures exhibiting lower values of k (Table V). Mean values of pulse maxima were generally indicative of values of k_{adj}. For example, a mean pulse maximum of 13.393 (arcsin-$\sqrt{\%\text{fissions/day}}$) corresponded to a mean k_{adj} of 0.0574, while a significantly lower (Student-Neuman-Keuls, $P < 0.05$) mean pulse maximum of 7.920 (arcsin-$\sqrt{\%\text{fissions/day}}$) accompanied a k_{adj} of 0.0278 (Table V). Likewise, significantly lower values of k_{adj} accompanied lower values for pulse minima (Table V).

Discussion

The distinction between k and k_{adj} is important for the analysis of fission activity in *H. luciae*. Fluctuations in population size and density are best considered in terms of k rather than k_{adj}, since periods of fission delay, as opposed to active periods of fission, are not easily distinguished in field studies. Moreover, fission-related morphological variability (Minasian, 1979) is best understood in relation to the total number of fissions over an entire interval (k), rather than the sustained or maximal rate of fission during part of that interval (k_{adj}). The parameter k_{adj} is a more accurate indication of active fission than is k. Hence, k_{adj} should be used to examine experimental effects upon fission rate. The calculation of k_{adj} requires that data be collected at frequent, preferably daily intervals.

The present study reveals three different effects of temperature upon fission activity. The first effect is upon fission rate. The Q_{10} value for k_{adj} in the 16 to 21° C range was three times greater than the Q_{10} value for the 21 to 26° C range. Sassaman and Mangum (1970) similarly found that oxygen consumption in *H. luciae* showed disproportionately large Q_{10} values over a range of 10 to 17.5° C. The second effect of temperature, a lengthening of the delay period, was also greatest below 21° C. Temperature also affected fission-pulse patterns: in cultures fed every second day, a 10° C decrease in culture temperature caused a 40% drop in the frequency of fission pulses, and a signficant decrease in values of pulse maxima and minima.

The great response of fission activity to small changes in temperature indicates that temperature is the foremost exogenous regulator of k. Miyawaki (1952) believed that a temperature threshold of approximately 15° C, below which no fission occurred, existed for *H. luciae*. He observed no longitudinal fission at temperatures below 20° C. The present study shows that values of k for this Florida clone decrease sharply as temperatures descend to 15° C, and in this respect agrees with Miyawaki's (1952) observations on Japanese *H. luciae*. However, a temperature threshold which limits fission in *H. luciae* is dependent upon other parameters which also affect k, such as food availability. Thus, k is best regarded as having a graded response to temperature, rather than an absolute threshold of 15° C.

The effect of feeding frequency upon k appears to be temperature-dependent. Although halving the feeding frequency resulted in reductions of k by only 4.5% at 26° C, and 8.9% at 21° C, a relatively large effect of decreased feeding frequency upon k occurred at 16° C, where the delay period was greatly lengthened. A temperature-dependent effect of feeding on the morphology of *H. luciae* reflects such interaction of effects on k (Minasian, 1979).

Exogenous factors determine sensitivity to mechanical stimuli which interrupt fission activity. Transferring anemones to new culture vessels (when setting up experimental cultures) imparted a mechanical disturbance, which caused little or no fission delay at high temperature and feeding frequency; but at low temperatures and feeding frequencies this delay may last for several weeks. Johnson and Shick (1977) demonstrated that fluctuating immersion-emersion cycles decrease fission activity in *H. luciae*. The mechanism underlying this effect may involve mechanically induced delays.

The synchrony of fission cessation following initial fission activity in low-temperature cultures (Fig. 1) is reminiscent of a synchronized cell culture (*e.g.*, Zeuthen and Scherbaum, 1954; Zeuthen, 1964), and implies the existence of relatively constant periods between fissions among individuals within the culture population. This long-term fission synchrony is most evident at low temperatures because periods between fission events are lengthened. When exogenous factors permit only short periods between fissions, such long-term synchronization is not observed. Studies on the lengths of inter-fission periods must be made on individual anemones to ascertain if inter-fission periods are relatively constant in duration, and how they change due to exogenous influences. The inter-fission period may simply be a delay in response to the previous fission event, which itself constitutes a mechanical disturbance.

The coincidence of fission pulses with feeding days, in cultures fed every second day, points to a regulatory role for other exogenous, and possibly endogenous factors. Photoperiodic and feeding-digestion cycles appear to be involved. For example, cultures of *H. luciae* are more active during dark portions of the photoperiodic cycle, and usually exhibit greater fission activity in the dark (Minasian, unpublished data). Similarly, Batham and Pantin (1950) observed pedal locomotory activity of the sea anemone, *Metridium senile,* to occur only at night. Although feeding causes long-term enhancement of fission, Torrey and Mery (1904) suggested that fission in *H. luciae* is inhibited by feeding. Thus, interaction of photoperiodic rhythms and short-term inhibition by feeding produces a 2-day pulse pattern in cultures fed at 2-day intervals: fission is inhibited during the first dark period subsequent to feeding, followed by a release from inhibition and increased fission activity during the second dark period after feeding. This inhibition of fission during hours subsequent to feeding explains the even-day duration of periods between fission-pulse maxima in cultures fed at 2-day intervals.

In cultures fed at 4-day intervals, periods between fission-pulse maxima are longer, with pulses of fission activity often beginning during the 24 hr subsequent to feeding. Minasian (1976) similarly observed that *H. luciae* undergo more synchronous fission activity in response to renewed feeding when starved for longer periods. Thus, cultures fed at different frequencies have inherent differences in the response of fission activity to feeding. If there exists an endogenous influence

on the fission-pulse pattern, it may permit synchronization of fission pulses with 2-day feeding intervals, but not with 4-day intervals.

The extreme sensitivity of fission activity to temperature indicates that the clone of *H. luciae* examined in these culture experiments must achieve recruitment primarily when temperatures exceed 20° C. Below 20° C, low-temperature inhibition is reinforced by tidal cycles, which can limit food availability and impart mechanical disturbance through immersion-emersion effects. Hence, below 20° C k will be small, and delays between major pulses of fission activity will be long. Previous studies (Shick and Lamb, 1977; Minasian 1979) have stated that the absence of gametogenesis (*i.e.,* absence of sexual reproduction) in *H. luciae* is associated with small size (and hence high k). Thus, the 15° to 20° C temperature range probably marks a crucial transition from small, sterile and strictly asexual anemones exhibiting high k, to larger, sexually reproductive anemones which exhibit only infrequent fission. Under present culture conditions, periodic, pulsed increments of fission activity are best interpreted in relation to two periodic, exogenous stimuli: photoperiod and feeding. In natural populations of *H. luciae*, periodic stimuli include photoperiod and tidal fluctuation, of which the latter involves both feeding and a mechanical (immersion-emersion) effect (Johnson and Shick, 1977). Therefore, it is possible that such pulses of fission activity have a phasic dependence upon tidal and photoperiodic cycles in intertidal populations of *H. luciae*. An investigation of fission activity under field conditions may elucidate this relationship.

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Summary

1. Permanent cultures of a clone of *H. luciae* from N. W. Florida were reared under different temperature and feeding regimes in order to identifiy and quantify parameters of asexual reproduction.

2. The principle components of fission activity include fission rate, a delay period following a mechanical disturbance, and periodic pulses of increased fission activity; all components are regulated by temperature and feeding frequency.

3. A distinction is made between fission rate including the delay period (k), and fission rate following the delay period (k_{adj}).

4. Fission rates (k_{adj}) ranged from 0.0162 (doubling time = 42.8 days) at 17° C to 0.0727 (doubling time = 9.5 days) at 26° C.

5. Temperature is the foremost regulator of k; the greatest influence of feeding frequency was upon periodic pulses of fission activity.

6. Culture data indicate that recruitment in natural populations of this clone is restricted by seasonal temperature; below 20° C there is a sharp reduction in k. It is suggested that inhibition of k by temperatures below 20° C favors a transition from asexual to sexual reproduction.

7. The pulsatile, periodic character of fission activity is prominent in laboratory cultures, and suggests that such activity in natural habitats may have a phasic dependence upon tidal and photoperiodic cycles.

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